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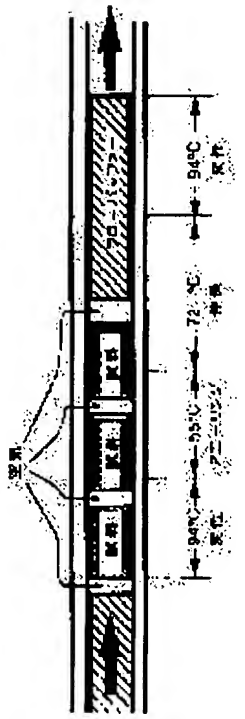
(54) METHOD FOR AMPLIFYING DNA AND DEVICE FOR AMPLIFYING DNA

(57)Abstract:

PURPOSE: To carry out PCR reaction in an accurate heating time by transporting a reaction solution comprising DNA polymerase, template DNA, primer DNA and dNTP as a mobile phase through a reaction tube heated from the outside.

CONSTITUTION: A reaction solution comprising DNA polymerase, template DNA, primer DNA and dNTP is used as a mobile phase and passed through a reaction tube, for example, controlled at 94° C, 55°C and 73°C. The three heating parts of the hydrocarbon tube are set at the outside and the temperature of the reaction solution moving in the reaction tube is regulated.



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CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS

[Translation done.]

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CLAIMS

[Claim(s)]
[Claim 1] DNA polymerase, template DNA, primer DNA, and the DNA magnification approach using an PCR reaction including the process to which it is made to move within [where the heating unit was prepared outside by making the reaction mixture containing dNTP into a mobile phase] a reaction.
[Claim 2] The approach containing two or more heating units adjusted by the temperature from which this heating unit differs according to claim 1.
[Claim 3] The approach containing three sorts of heating units adjusted by about 94 degrees C, about 55 degrees C, and about 73 degrees C according to claim 2.
[Claim 4] The method according to claim 3 of moving this reaction mixture within a reaction with constant speed.
[Claim 5] The DNA amplifying device equipped with the power unit for moving the heating unit and this reaction mixture which were prepared in the outside of the coil for moving DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP inside, and this coil.
[Claim 6] Furthermore, equipment according to claim 5 with which a buffer tank, reaction mixture induction, and a reaction mixture stripping section were prepared.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the DNA magnification approach using the polymerase chain reaction method which amplifies the gene DNA of a minute amount efficiently. It is related with the equipment used for DNA polymerase, template DNA, primer DNA, the DNA magnification approach of making it moving within a reaction by making the reaction mixture containing dNTP into a mobile phase, and this approach, in more detail.
[Description of the Prior Art] As an approach of amplifying minute amount DNA efficiently, it is a polymerase chain reaction method (polymerase chain reaction, PCR method). It is known (U.S. Pat. No. 4,683,202 specification). This approach is an approach that the purpose DNA can be made to amplify exponentially, by carrying out thermal denaturation of the target DNA, carrying out annealing of two kinds of primers to DNA of the obtained single strand, and repeating a cycle called composition of the complementary DNA by annealing and DNA polymerase of a primer, after making DNA polymerase act after that, compounding the double stranded DNA, carrying out thermal denaturation of this double stranded DNA further and obtaining a single stranded DNA. Generally the PCR method is performed by repeating a cycle including the process which makes the double stranded DNA dissociate at about 94 degrees C, the process which carries out annealing of the primer at about 55 degrees C, and the process which reproduces a complementary strand at about 72 degrees C using heat-resistant DNA polymerase. Therefore, management of the temperature in each process and reaction time is important.
[0002] The Eppendorf mold sample tube which contains a reaction mixture as equipment which performs the PCR method automatically is inserted in the well in which it was prepared by the block made from aluminum, and the equipment which reacts by changing the temperature of this aluminum block using a heater and a condensator is known (DNA Thermal Cycler, Perkin-Elmer Cetus Instruments). With this equipment, in order to promote a heat block and heat conduction between sample tubes, the device of filling up with oil the gap formed with a well and a sample tube is given. However, since it began to have changed the temperature of a heat block also with this means, by the time the temperature of the reaction mixture in a sample tube reached the purpose temperature, there were 10 thru/or delay for about 30 seconds. For this reason, the reaction time of a denaturation process (about 94 degrees C) was extended according to such time delay, deactivation of an enzyme was caused, and there was a problem to which magnification effectiveness falls as a result. Moreover, since the waterdrop inside evaporation of moisture or the lid of a sample tube is formed in order to raise the temperature of reaction mixture to near the boiling point of water, in order to prevent this, the laminating of the oil for antiflashing needed to be carried out to the reaction mixture front face in a sample tube. Furthermore, when performing a magnification reaction using many samples, each sample needed to be made to prepare and react to a separate sample tube, and actuation was complicated. Moreover, the PCR equipment in which you make it the bucket which fixed the sample tube mechanically immersed one after another to the thermostat set as three different temperature is known (ThermalSequencer, Iwaki). However, this reactor had the fault of being large-scale.
[0003] Therefore, this invention aims at offering the equipment used for the DNA magnification approach and this approach using the polymerase chain reaction approach without the above-mentioned fault.
[Means for Solving the Problem] In performing a polymerase chain reaction reaction, as a result of trying

hard wholeheartedly that the above-mentioned technical problem should be solved, by making it move within [where the heating unit was prepared outside] a reaction, using DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP as a mobile phase, this invention person can adjust strictly the reaction temperature and reaction time of this reaction mixture, and came to complete a header and this invention for the ability of a polymerase chain reaction reaction to be performed efficiently. That is, this invention offers the equipment used for the DNA magnification approach and this approach using the PCR reaction approach including the process to which it is made to move within [where the heating unit was prepared outside by making DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP into a mobile phase] a reaction.

[0004] DNA polymerase, template DNA, primer DNA, and dNTP are contained in the reaction mixture used for the approach of this invention. Template DNA may be DNA used as the purpose of magnification, and may be which DNA of a natural mold or a non-natural mold. Template DNA is prepared by SDSI protease K processing from a cell by the approach obvious to this contractor. Moreover, DNA directly extracted from the colony can also be used. usually, template DNA -- about 0.01 to 100 PM -- what is necessary is just to use it by the concentration of 0.1 - 10PM preferably As DNA polymerase, what kind of obvious DNA polymerase may be used for this contractor as DNA polymerase used for DNA magnification. Heat-resistant DNA polymerase can be mentioned as DNA polymerase used suitable for the approach of this invention. For example, thermotolerant enzyme It is desirable to use Tth (thermus thermophilus) polymerase, Taq (thermus aquaticus) polymerase, etc. these DNA polymerase -- usually -- 10-40U/ml -- it is preferably used by 20U/ml concentration.

[0005] Although what is necessary is just to use an obvious thing for this contractor as primer DNA as primer DNA which can be used for the DNA amplifying method, the synthetic DNA of 20-mer extent can be used, for example. primer DNA -- for example, abbreviation 100 - 1,000 nM -- desirable -- What is necessary is just to use it by the concentration of 200 - 500 nM. primer DNA -- a DNA automatic composition machine -- **** -- this contractor can manufacture easily if needed by things. As for above-mentioned DNA polymerase and above-mentioned primer DNA, in performing the approach of this invention, being chosen that magnification effectiveness should be made max is desirable, but according to this contractor, such selection is made easily. Moreover, in this specification, although defined as dNTP being the mixture of the rate of the arbitration of dATP, dGTP, dTTP, and dCTP, it is desirable to use the equivalent mixture of these four sorts of nucleotide TORIRIN acids. For example, four sorts of nucleotide TORIRIN acids are 10-100 in reaction mixture. muM Containing by concentration is desirable. Furthermore, as for the reaction mixture used for the approach of this invention, it is desirable that the buffer other than the above component is included. Since magnification effectiveness changes with classes of buffer solution generally used for a reaction, as for the DNA polymerase used for the PCR method, it is desirable to choose a suitable buffer according to the class of DNA polymerase to be used. Although what is necessary is just to adjust suitably DNA polymerase, template DNA, primer DNA, the blending ratio of coal of dNTP, and the concentration of a buffer according to the purpose, such adjustment is easily made by this contractor.

[0006] In performing an PCR reaction in the above-mentioned reaction mixture, the approach of this invention is the DNA magnification approach characterized by making it move within [where heating was formed outside] a reaction, using this reaction mixture as a mobile phase. As long as it was formed of the member which does not affect the PCR reaction performed inside a coil as a coil used for the approach of this invention, what kind of thing may be used. For example, the coil formed with Teflon, polyethylene, glass, stainless steel, etc. can be mentioned. the bore of a coil -- 0.02 - 1 mm -- desirable -- 0.05 - 0.5 mm -- then, it is good. That what is necessary is just to choose the bore of a coil suitably according to the capacity of the reaction mixture processed at once, if the coil of the diameter of minute is used, the DNA magnification reaction of a minute amount can be performed. For example, when performing the reaction of a super-minute amount, a glass capillary tube can be used as a coil. Unless heat conduction from the heating unit installed in the outside of a coil to the reaction mixture of the section of a reaction falls remarkably, what kind of thing is sufficient as reaction wall thickness. therefore -- although the outer diameter of a coil changes with classes of member used -- general -- 0.1 - 2 mm -- desirable -- 0.2 - 1.5 mm -- then, it is good. the case where the coil made from Teflon is used -- desirable -- a bore 0.3 - 0.7 mm, and an outer diameter 1.0 - 1.8 mm -- what is necessary is just to use a coil with a bore [of 0.5mm], and an outer diameter of 1.5mm preferably especially

[0007] What is necessary is just to introduce continuously liquid fluids, such as gas fluids, such as air, nitrogen gas, and argon gas, distilled water, and the buffer solution, within a reaction, after introducing the reaction mixture of the specified quantity within a reaction, in order to move the above-mentioned reaction mixture within [this] a reaction. In introducing this fluid continuously, a peristaltic pump, the pump for high performance chromatography, etc. are used suitably. What is necessary is for a micro syringe etc. just to perform using the injector for high speed liquid chromatographies, in order to introduce reaction mixture into a coil. Moreover, what is necessary is to introduce gas fluids, oil, etc., such as air, nitrogen gas, and argon gas, and just to introduce the reaction mixture of the specified quantity further, after introducing the reaction mixture of the specified quantity within a reaction in processing continuously the reaction mixture from which plurality differs within a reaction. By repeating this actuation, it can be made to be able to convey within a reaction so that two or more reaction mixture may not be mixed, and the reaction mixture containing a different component can be processed to coincidence. If the conveyance condition of the reaction mixture within a reaction is explained referring to drawing 1 , installation of reaction mixture will be preceded. DNA polymerase, template DNA, primer DNA, and the buffer solution (flow buffer) that does not contain dNTP are introduced within a reaction. The reaction mixture of a small amount of air and the specified quantity is repeated 3 times by turns after that, it introduces into a coil, finally a flow buffer is introduced within a reaction continuously, and reaction mixture is moved within a reaction.

[0008] By the approach of this invention, it faces moving reaction mixture within a reaction as mentioned above, and the temperature of the reaction mixture which moves within a reaction is adjusted by the heating unit prepared in the outside of a coil. Generally the PCR method is performed considering a cycle including the process (denaturation) which makes the double stranded DNA usually dissociate at about 94 degrees C, the process (annealing) which carries out annealing of the primer at about 55 degrees C, and the process (expanding) which reproduces a complementary strand at about 72 degrees C using heat-resistant DNA polymerase as a base unit. For this reason, in the approach of this invention, the heating unit for denaturation processes (94 degrees C) adjusted by the three above-mentioned sorts of temperature on the outside of a coil, respectively, the heating unit for annealing processes (55 degrees C), and the heating unit for expanding processes (72 degrees C) are prepared one by one preferably, and predetermined time warming of the reaction mixture which moves within a reaction should just be made to be carried out at the three above-mentioned sorts of different temperature. What is necessary is just to set up more highly a little the temperature of a heating unit which was prepared in the outside of a coil depending on the quality of the material of a coil in such a case rather than whenever [reaction liquid temperature], since thermal conductivity may not be enough. Usually, since reaction mixture is conveyed so that it may move within a reaction with constant speed, heating time can be adjusted according to the die length of the heating unit prepared in the coil. Moreover, the die length of a heating unit is fixed, the passing speed of reaction mixture may be changed, and heating time may be adjusted. Both die length of a heating unit and passing speed of reaction mixture may be changed, and heating time may be adjusted.

[0009] If it explains referring to drawing 1 , the approach of this invention can adjust to 3:4:6 the die length of a heating unit (94 degrees C prepared in the outside of a coil in the denaturation process when an annealing process was performed at 55 degrees C and 72 degrees C performed 2 minutes and an expanding process for 30 seconds per minute at 94 degrees C for 3 minutes, 55 degrees C, and 72 degrees C), for example, and can perform it by conveying reaction mixture with constant speed. Although what kind of thing may be used as long as it can supply fixed temperature to a coil as a heating unit prepared in the outside of a coil, a heat block, a thermostat, heating air, etc. are used suitably, for example. For example, what is necessary is to use three sorts of thermostats set as 94 degrees C, 55 degrees C, and 72 degrees C, and just to immerse the coil of predetermined die length in warm water or oil in a thermostat etc., in using a thermostat. The PCR method makes a basic cycle a denaturation process, an above-mentioned annealing process, and an above-mentioned expanding process, and is usually performed by repeating this cycle 25 to 35 times. What is necessary is to repeat and prepare a heating unit (94 degrees C, 55 degrees C, and 72 degrees C), and just to heat-treat the required number of cycles, in using the reactor of a linear model shown in drawing 1 . When repeating many cycles, it can amplify efficiently by processing a coil for example, into a spiral mold, repeating the heating unit for denaturation processes, the heating unit for annealing processes, and the heating unit for expanding

processes, and making it pass. For example, when using a thermostat as a heating unit, only the number corresponding to the count of a reaction should be repeatedly immersed in three kinds of thermostats in the coil processed into the spiral mold as shown in drawing 2 . By preparing a fraction collector in the end of a coil, the reaction mixture containing DNA amplified by PCR is efficiently recoverable. Moreover, detecting elements, such as for example, an ultraviolet-rays detector, may be prepared in the middle of a coil, and you may act as the monitor of the magnification process.

[0010] According to another mode of this invention, the DNA amplifying device for performing the DNA magnification approach of this invention is offered. The equipment of this invention is a DNA amplifying device equipped with the power unit 3 for moving the heating unit 2 and this reaction mixture which were prepared in the outside of the coil 1 for DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP to make it move inside, and this coil within [this] a reaction, and this equipment is equipment with which the buffer tank 6, the reaction mixture induction 4, and the reaction mixture stripping section 5 were formed still more preferably. What is necessary is to be able to use the above-mentioned thing as a coil and a heating unit, and just to use an HPLC pump etc. as a power unit, for example. What is necessary is just to use the injector for HPLC etc. for a reaction mixture stripping section for a fraction collector etc. as reaction mixture induction, for example. Although one example of the equipment of this invention is shown in drawing 2 , the equipment of this invention is not limited to this equipment.

[0011]

[Effect of the Invention] According to the approach of this invention, PCR Noriyuki ****** is made in very exact heating time, and DNA magnification can be performed efficiently. By the approach of this invention, there is also no deactivation of the enzyme by the delay of heating time, and magnification effectiveness does not fall. Moreover, since there is no waterdrop formation by the inside of evaporation of moisture or the lid of a sample tube, it is not necessary to use the oil for antiflashing. Furthermore, since a magnification reaction can be performed to coincidence using many samples, it is useful.

[Example] Although an example explains this invention still more concretely below, this invention is not limited to these examples.

[0012] The approach of this invention was enforced using the equipment shown in drawing 2 . The Teflon coil (the bore of 0.5mm, 1.5mm of appearances) was used as a coil 1, and the thermostat which filled the warm water adjusted by 94 degrees C, 55 degrees C, and 73 degrees C as a heating unit 2 was used. Tietech thermostat MINDA was used for each thermostat as a thermostat. The end of a coil 1 is connected to the power unit 3, and the reaction mixture induction 4 is formed in the middle of the coil from a power unit to a heating unit. The flow buffer is supplied from the buffer tank 6. In this example, the injector for HPLC made from a LEO dyne was used as Jasco 1 [HPLC pump BIP-] and reaction mixture induction 4 as a power unit 3. The coil 1 with which sequential immersion is carried out to 94-degree C warm water by the die length of 300 mm in 200 mm and 73-degree C warm water at 150 mm and 55-degree C warm water, and the coil 1 came out of 72 more-degree C warm water to it in the heating unit 2 is processed into the spiral mold so that sequential immersion may be carried out by the again same die length as warm water (94 degrees C, 55 degrees C, and 73 degrees C). A 94-degree C thermostat is a tub for making the double stranded DNA dissociate, a 55-degree C thermostat is a tub for carrying out annealing of the primer DNA to DNA dissociated to the single strand, and a 73-degree C tub is a tub which makes the 2nd chain reproduce from DNA which primer DNA combined. The reaction mixture inside a coil and a coil is also held at the same temperature. The coil 1 forms the spiral of 22 rolls on the whole, and repeat processing of the reaction mixture which moves in the interior of a coil 1 is carried out 22 times by the 94 degrees C -> 55 degrees C -> 73 degrees C temperature cycle. The coil 1 which came out of the 73-degree C heating unit 2 is connected to the fraction collector as a reaction mixture stripping section 5, and recovery of reaction mixture is performed here.

[0013] Above equipment was used and the flow buffer was poured into the interior of a coil 1. After stabilizing the flow rate of a flow buffer, and the temperature of a heating unit, a micro syringe is used, and it is air 5microl. It poured in from the reaction mixture induction 4. 5micro of after that reaction mixture I And air 5microl Sequential impregnation was carried out, the flow buffer was poured in continuously further again, and reaction mixture was moved to the heating unit. Reaction mixture and a buffer presentation are as being shown in the following table 1. Fractionation of the reaction mixture after heat-treatment was carried out by the fraction collector, and the product was checked by agarose gel

electrophoresis 1%.
[Table 1]
Tth Polymerase: Flow buffer : 67 mM Tris and hydrochloric-acid (pH8.8) / 16.6 mM2(NH4) SO4/ 6.7 mM MgCl2 Reaction buffer : 67 mM Tris and hydrochloric-acid (pH 8.8) / 16.6 mM2(NH4) SO4/ 6.7 mM MgCl2/10 mM 2-ME/0.2 mM dNTPs Taq Polymerase: Flow buffer : 10 mM Tris and hydrochloric-acid (pH 8.3) / 50 mM KCl/ 2 mM MgCl2 Reaction buffer : 10 mM Tris and hydrochloric-acid (pH 8.3) / 50 mM KCl/ 2 mM MgCl2 / 0.2 mM dNTPs primer DNA (they are after composition and OPC with an ABIDNA composition machine it refines by the cartridge) : UN-24 : 5'-CGCCAGGGTTTTCCCAGTCACGAC-3' UNR-24 : 5'-TTTCACACAGGAAACAGCTATGAC-3' Template DNA: pNF-1 : 1.2 kb PCR Product pUCtDHFR : 0.7 kb PCR Product pBluescript KS+ : 0.23 kb PCR Product [0014] It is 20 as example of trial 1 template DNA. pNF-1 of ng/mu l (10 nM) It used and the approach of this invention was enforced using two kinds of DNA polymerase. A result is shown in Table 2. The number of front Naka and + shows extent of magnification, and it is shown that extent of magnification is so high that there is many +.
[Table 2]
No. Polymerase The rate of flow Linear velocity The processing time Amplification degree mul/min. mm/min. 94 **-55 degree-C-73 degree C Example 1 Tth 20 102 1'30" - 2' -3' Example 2 of +++ Taq 20 102 1'30" - 2' Example of example of -3' ++ 3 Tth 40 204 45 "-1'-1'30" ++ 4Tth 60 306 30" [[0015]] - 40" - 1'+ It is pNF-1 as example of trial 2 template DNA. It used (they are 100 ** and the thing processed for 5 minutes at 0.5% Triton X-100), the concentration of template DNA was changed, and the approach of this invention was enforced. As polymerase Tth polymerase is used and it is the rate of flow. 40 mul/min., linear velocity 204 mm/min. and processing-time (94 **-55 degree-C-73 degree C) 45" [It carried out.] - 1' - 1'30" The same experiment was conducted using thermal SAIKURA of SHITASU as an example of a comparison. A result is shown in Table 3.
[Table 3]
No. Template DNA concentration Amplification degree Ng/mul Example 5 1 + The example 6 of ++ 0.1 + The example 1 of a comparison 0.02 + The example 2 of a comparison 0.2 + The example 3 of a comparison 0.4 The example 4 of ++ comparison 1.0 The example 5 of a +++ comparison 2.0 +++ As example of trial 3 template DNA 1 Ng/mul pNF-1 (1.2 kbp fragmentation), pBluescriptKS+ (0.23 kbp fragmentation) -- and -- pUCtDHFR (0.7 kbp fragmentation) It used and consecutive processing was performed. pNF-1 Included reaction mixture 5microl and pBluescript KS+ included reaction mixture 5microl -- and -- pUCtDHFR Included reaction mixture 5microl It **********ed at intervals of 10 minutes. It dissociated with air between each reaction mixture. As DNA polymerase Tth polymerase is used and they are rate-of-flow 40microl/min. and linear velocity. 204 mm/min. and processing-time (94 **-55 degree-C-73 degree C) 45" [It carried out.] - 1' - 1'30" Consequently, there was almost no cross contamination in each PCR, and good magnification was possible for it.

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TECHNICAL PROBLEM

[Description of the Prior Art] As an approach of amplifying minute amount DNA efficiently, it is a polymerase chain reaction method (polymerase chain reaction, PCR method). It is known (U.S. Pat. No. 4,683,202 specification). This approach is an approach that the purpose DNA can be made to amplify exponentially, by carrying out thermal denaturation of the target DNA, carrying out annealing of two kinds of primers to DNA of the obtained single strand, and repeating a cycle called composition of the complementary DNA by annealing and DNA polymerase of a primer, after making DNA polymerase act after that, compounding the double stranded DNA, carrying out thermal denaturation of this double stranded DNA further and obtaining a single stranded DNA. Generally the PCR method is performed by repeating a cycle including the process which makes the double stranded DNA dissociate at about 94 degrees C, the process which carries out annealing of the primer at about 55 degrees C, and the process which reproduces a complementary strand at about 72 degrees C using heat-resistant DNA polymerase. Therefore, management of the temperature in each process and reaction time is important.
[0002] The Eppendorf mold sample tube which contains a reaction mixture as equipment which performs the PCR method automatically is inserted in the well in which it was prepared by the block made from aluminum, and the equipment which reacts by changing the temperature of this aluminum block using a heater and a condensator is known (DNA Thermal Cycler, Perkin-Elmer Cetus Instruments). With this equipment, in order to promote a heat block and heat conduction between sample tubes, the device of filling up with oil the gap formed with a well and a sample tube is given. However, since it began to have changed the temperature of a heat block also with this means, by the time the temperature of the reaction mixture in a sample tube reached the purpose temperature, there were 10 thru/or delay for about 30 seconds. For this reason, the reaction time of a denaturation process (about 94 degrees C) was extended according to such time delay, deactivation of an enzyme was caused, and there was a problem to which magnification effectiveness falls as a result. Moreover, since the waterdrop inside evaporation of moisture or the lid of a sample tube is formed in order to raise the temperature of reaction mixture to near the boiling point of water, in order to prevent this, the laminating of the oil for antiflashing needed to be carried out to the reaction mixture front face in a sample tube. Furthermore, when performing a magnification reaction using many samples, each sample needed to be made to prepare and react to a separate sample tube, and actuation was complicated. Moreover, the PCR equipment in which you make it the bucket which fixed the sample tube mechanically immersed one after another to the thermostat set as three different temperature is known (ThermalSequencer, Iwaki). However, this reactor had the fault of being large-scale.
[0003] Therefore, this invention aims at offering the equipment used for the DNA magnification approach and this approach using the polymerase chain reaction approach without the above-mentioned fault.

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MEANS

[Means for Solving the Problem] In performing a polymerase chain reaction reaction, as a result of trying hard wholeheartedly that the above-mentioned technical problem should be solved, by making it move within [where the heating unit was prepared outside] a reaction, using DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP as a mobile phase, this invention person can adjust strictly the reaction temperature and reaction time of this reaction mixture, and came to complete a header and this invention for the ability of a polymerase chain reaction reaction to be performed efficiently. That is, this invention offers the equipment used for the DNA magnification approach and this approach using the PCR reaction approach including the process to which it is made to move within [where the heating unit was prepared outside by making DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP into a mobile phase] a reaction.
[0004] DNA polymerase, template DNA, primer DNA, and dNTP are contained in the reaction mixture used for the approach of this invention. Template DNA may be DNA used as the purpose of magnification, and may be which DNA of a natural mold or a non-natural mold. Template DNA is prepared by SDSI protease K processing from a cell by the approach obvious to this contractor. Moreover, DNA directly extracted from the colony can also be used. usually, template DNA -- about 0.01 to 100 PM -- what is necessary is just to use it by the concentration of 0.1 - 10PM preferably As DNA polymerase, what kind of obvious DNA polymerase may be used for this contractor as DNA polymerase used for DNA magnification. Heat-resistant DNA polymerase can be mentioned as DNA polymerase used suitable for the approach of this invention. For example, thermotolerant enzyme It is desirable to use Tth (thermus thermophilus) polymerase, Taq (thermus aquaticus) polymerase, etc. these DNA polymerase -- usually -- 10-40U/ml -- it is preferably used by 20U/ml concentration.
[0005] Although what is necessary is just to use an obvious thing for this contractor as primer DNA as primer DNA which can be used for the DNA amplifying method, the synthetic DNA of 20-mer extent can be used, for example. primer DNA -- for example, abbreviation 100 - 1,000 nM -- desirable -- What is necessary is just to use it by the concentration of 200 - 500 nM. primer DNA -- a DNA automatic composition machine -- **** -- this contractor can manufacture easily if needed by things. As for above-mentioned DNA polymerase and above-mentioned primer DNA, in performing the approach of this invention, being chosen that magnification effectiveness should be made max is desirable, but according to this contractor, such selection is made easily. Moreover, in this specification, although defined as dNTP being the mixture of the rate of the arbitration of dATP, dGTP, dTTP, and dCTP, it is desirable to use the equivalent mixture of these four sorts of nucleotide TORIRIN acids. For example, four sorts of nucleotide TORIRIN acids are 10-100 in reaction mixture. muM Containing by concentration is desirable. Furthermore, as for the reaction mixture used for the approach of this invention, it is desirable that the buffer other than the above component is included. Since magnification effectiveness changes with classes of buffer solution generally used for a reaction, as for the DNA polymerase used for the PCR method, it is desirable to choose a suitable buffer according to the class of DNA polymerase to be used. Although what is necessary is just to adjust suitably DNA polymerase, template DNA, primer DNA, the blending ratio of coal of dNTP, and the concentration of a buffer according to the purpose, such adjustment is easily made by this contractor.
[0006] In performing an PCR reaction in the above-mentioned reaction mixture, the approach of this invention is the DNA magnification approach characterized by making it move within [where heating was

formed outside] a reaction, using this reaction mixture as a mobile phase. As long as it was formed of the member which does not affect the PCR reaction performed inside a coil as a coil used for the approach of this invention, what kind of thing may be used. For example, the coil formed with Teflon, polyethylene, glass, stainless steel, etc. can be mentioned. the bore of a coil -- 0.02 - 1 mm -- desirable -- 0.05 - 0.5 mm -- then, it is good. That what is necessary is just to choose the bore of a coil suitably according to the capacity of the reaction mixture processed at once, if the coil of the diameter of minute is used, the DNA magnification reaction of a minute amount can be performed. For example, when performing the reaction of a super-minute amount, a glass capillary tube can be used as a coil. Unless heat conduction from the heating unit installed in the outside of a coil to the reaction mixture of the section of a reaction falls remarkably, what kind of thing is sufficient as reaction wall thickness. therefore -- although the outer diameter of a coil changes with classes of member used -- general -- 0.1 - 2 mm -- desirable -- 0.2 - 1.5 mm -- then, it is good. the case where the coil made from Teflon is used -- desirable -- a bore 0.3 - 0.7 mm, and an outer diameter 1.0 - 1.8 mm -- what is necessary is just to use a coil with a bore [of 0.5mm], and an outer diameter of 1.5mm preferably especially

[0007] What is necessary is just to introduce continuously liquid fluids, such as gas fluids, such as air, nitrogen gas, and argon gas, distilled water, and the buffer solution, within a reaction, after introducing the reaction mixture of the specified quantity within a reaction, in order to move the above-mentioned reaction mixture within [this] a reaction. In introducing this fluid continuously, a peristaltic pump, the pump for high performance chromatography, etc. are used suitably. What is necessary is for a micro syringe etc. just to perform using the injector for high speed liquid chromatographies, in order to introduce reaction mixture into a coil. Moreover, what is necessary is to introduce gas fluids, oil, etc., such as air, nitrogen gas, and argon gas, and just to introduce the reaction mixture of the specified quantity further, after introducing the reaction mixture of the specified quantity within a reaction in processing continuously the reaction mixture from which plurality differs within a reaction. By repeating this actuation, it can be made to be able to convey within a reaction so that two or more reaction mixture may not be mixed, and the reaction mixture containing a different component can be processed to coincidence. If the conveyance condition of the reaction mixture within a reaction is explained referring to drawing 1 , installation of reaction mixture will be preceded. DNA polymerase, template DNA, primer DNA, and the buffer solution (flow buffer) that does not contain dNTP are introduced within a reaction. The reaction mixture of a small amount of air and the specified quantity is repeated 3 times by turns after that, it introduces into a coil, finally a flow buffer is introduced within a reaction continuously, and reaction mixture is moved within a reaction.

[0008] By the approach of this invention, it faces moving reaction mixture within a reaction as mentioned above, and the temperature of the reaction mixture which moves within a reaction is adjusted by the heating unit prepared in the outside of a coil. Generally the PCR method is performed considering a cycle including the process (denaturation) which makes the double stranded DNA usually dissociate at about 94 degrees C, the process (annealing) which carries out annealing of the primer at about 55 degrees C, and the process (expanding) which reproduces a complementary strand at about 72 degrees C using heat-resistant DNA polymerase as a base unit. For this reason, in the approach of this invention, the heating unit for denaturation processes (94 degrees C) adjusted by the three above-mentioned sorts of temperature on the outside of a coil, respectively, the heating unit for annealing processes (55 degrees C), and the heating unit for expanding processes (72 degrees C) are prepared one by one preferably, and predetermined time warming of the reaction mixture which moves within a reaction should just be made to be carried out at the three above-mentioned sorts of different temperature. What is necessary is just to set up more highly a little the temperature of a heating unit which was prepared in the outside of a coil depending on the quality of the material of a coil in such a case rather than whenever [reaction liquid temperature], since thermal conductivity may not be enough. Usually, since reaction mixture is conveyed so that it may move within a reaction with constant speed, heating time can be adjusted according to the die length of the heating unit prepared in the coil. Moreover, the die length of a heating unit is fixed, the passing speed of reaction mixture may be changed, and heating time may be adjusted. Both die length of a heating unit and passing speed of reaction mixture may be changed, and heating time may be adjusted.

[0009] If it explains referring to drawing 1 , the approach of this invention can adjust to 3:4:6 the die length of a heating unit (94 degrees C prepared in the outside of a coil in the denaturation process when

an annealing process was performed at 55 degrees C and 72 degrees C performed 2 minutes and an expanding process for 30 seconds per minute at 94 degrees C for 3 minutes, 55 degrees C, and 72 degrees C), for example, and can perform it by conveying reaction mixture with constant speed. Although what kind of thing may be used as long as it can supply fixed temperature to a coil as a heating unit prepared in the outside of a coil, a heat block, a thermostat, heating air, etc. are used suitably, for example. For example, what is necessary is to use three sorts of thermostats set as 94 degrees C, 55 degrees C, and 72 degrees C, and just to immerse the coil of predetermined die length in warm water or oil in a thermostat etc., in using a thermostat. The PCR method makes a basic cycle a denaturation process, an above-mentioned annealing process, and an above-mentioned expanding process, and is usually performed by repeating this cycle 25 to 35 times. What is necessary is to repeat and prepare a heating unit (94 degrees C, 55 degrees C, and 72 degrees C), and just to heat-treat the required number of cycles, in using the reactor of a linear model shown in drawing 1 . When repeating many cycles, it can amplify efficiently by processing a coil for example, into a spiral mold, repeating the heating unit for denaturation processes, the heating unit for annealing processes, and the heating unit for expanding processes, and making it pass. For example, when using a thermostat as a heating unit, only the number corresponding to the count of a reaction should be repeatedly immersed in three kinds of thermostats in the coil processed into the spiral mold as shown in drawing 2 . By preparing a fraction collector in the end of a coil, the reaction mixture containing DNA amplified by PCR is efficiently recoverable. Moreover, detecting elements, such as for example, an ultraviolet-rays detector, may be prepared in the middle of a coil, and you may act as the monitor of the magnification process.

[0010] According to another mode of this invention, the DNA amplifying device for performing the DNA magnification approach of this invention is offered. The equipment of this invention is a DNA amplifying device equipped with the power unit 3 for moving the heating unit 2 and this reaction mixture which were prepared in the outside of the coil 1 for DNA polymerase, template DNA, primer DNA, and the reaction mixture containing dNTP to make it move inside, and this coil within [this] a reaction, and this equipment is equipment with which the buffer tank 6, the reaction mixture induction 4, and the reaction mixture stripping section 5 were formed still more preferably. What is necessary is to be able to use the above-mentioned thing as a coil and a heating unit, and just to use an HPLC pump etc. as a power unit, for example. What is necessary is just to use the injector for HPLC etc. for a reaction mixture stripping section for a fraction collector etc. as reaction mixture induction, for example. Although one example of the equipment of this invention is shown in drawing 2 , the equipment of this invention is not limited to this equipment.

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the conceptual diagram of the DNA amplifying device for enforcing the approach of this invention.
[Drawing 2] It is drawing showing the DNA amplifying device for enforcing the approach of this invention.
[Description of Notations]
1 Coil
2 Heating Unit
3 Power Unit
4 Reaction Mixture Induction
5 Reaction Mixture Stripping Section
6 Buffer Tank

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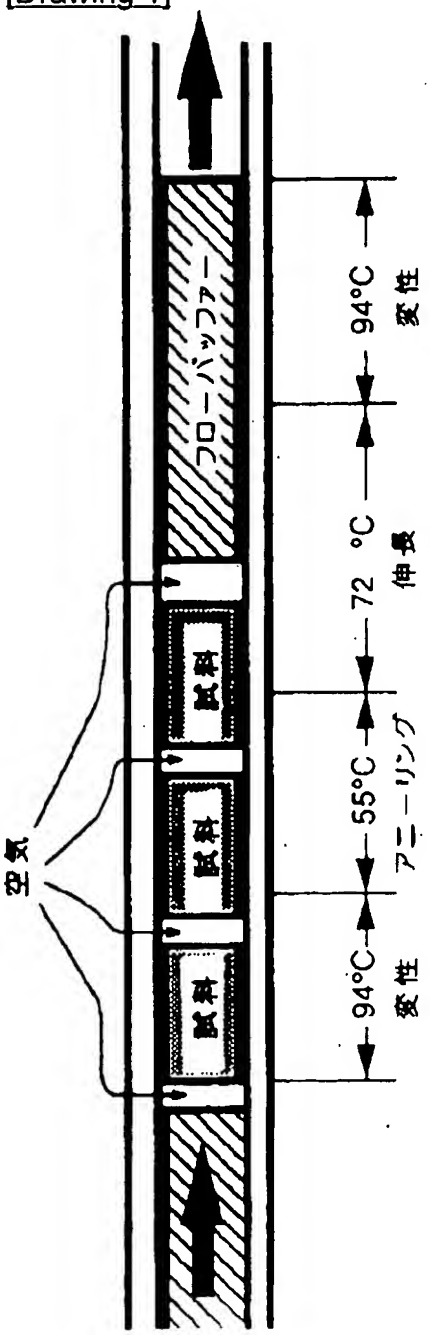
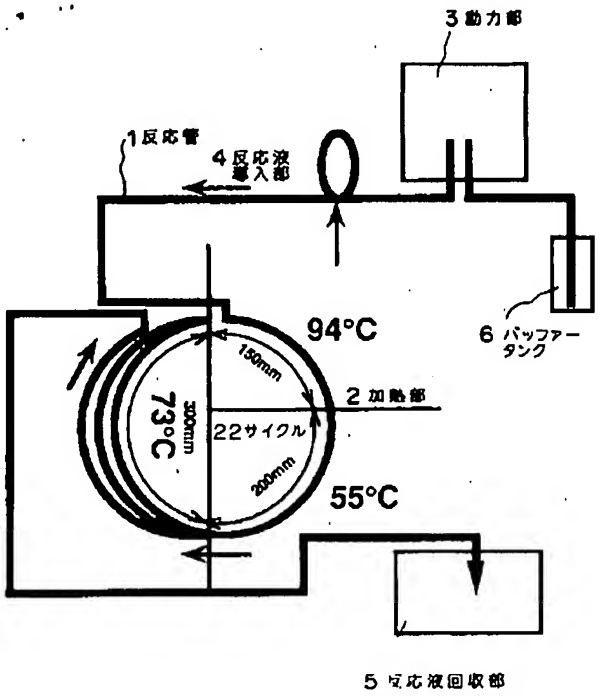
DRAWINGS

[Drawing 1]



[Drawing 2]



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